VOLUME 30 · NUMBER 13 · MAY 1 2012

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Phase II Study of Bevacizumab in Patients With HIV-Associated Kaposi's Sarcoma Receiving Antiretroviral Therapy

Thomas S. Uldrick, Kathleen M. Wyvill, Pallavi Kumar, Deirdre O'Mahony, Wendy Bernstein, Karen Aleman, Mark N. Polizzotto, Seth M. Steinberg, Stefania Pittaluga, Vickie Marshall, Denise Whitby, Richard F. Little, and Robert Yarchoan

Thomas S. Uldrick, Kathleen M. Wyvill, Pallavi Kumar, Deirdre O'Mahony, Wendy Bernstein, Karen Aleman, Mark N. Polizzotto, Seth M. Steinberg, Stefania Pittaluga, Richard F. Little, and Robert Yarchoan, Center for Cancer Research, National Cancer Institute (NCI), Bethesda; Vickie Marshall and Denise Whitby, SAIC-Frederick, NCI, Frederick, MD.

Submitted September 22, 2011; accepted January 6, 2012; published online ahead of print at www.jco.org on March 19, 2012.

Supported in part by the Intramural Research Program, National Cancer Institute (NCI), National Institutes of Health (NIH). Additional funding provided by the NCI, NIH, under Contract No. HHSN261200800001E.

Presented in part at the 17th Annual Conference on Retroviruses and Opportunistic Infections, February 16-19, 2010, San Francisco, CA.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

Corresponding author: Robert Yarchoan, MD, 10 Center Dr, Room 6N106, MSC 1868, Bethesda, MD 20892-1868; e-mail: Robert.Yarchoan@nih.gov.

0732-183X/12/3013-1476/$20.00

DOI: 10.1200/JCO.2011.39.6853

ABSTRACT

Purpose
Alternatives to cytotoxic agents are desirable for patients with HIV-associated Kaposi's sarcoma (KS). Vascular endothelial growth factor-A (VEGF-A) contributes to KS pathogenesis. We evaluated the humanized anti–VEGF-A monoclonal antibody, bevacizumab, in patients with HIV-KS.

Patients and Methods
Patients with HIV-KS who either experienced progression while receiving highly active antiretroviral therapy (HAART) for at least 1 month or did not regress despite HAART for at least 4 months were administered bevacizumab 15 mg/kg intravenously on days 1 and 8 and then every 3 weeks. The primary objective was assessment of antitumor activity using modified AIDS Clinical Trial Group (ACTG) criteria for HIV-KS. HIV-uninfected patients were also eligible and observed separately.

Results
Seventeen HIV-infected patients were enrolled. Fourteen patients had been receiving effective HAART for at least 6 months (median, 1 year). Thirteen patients had advanced disease (ACTG T_1), 13 patients had received prior chemotherapy for KS, and seven patients had CD4 count less than 200 cells/μL. Median number of cycles was 10 (range, 1 to 37 cycles); median follow-up was 8.3 months (range, 3 to 36 months). Of 16 assessable patients, best tumor responses observed were complete response (CR) in three patients (19%), partial response (PR) in two patients (12%), stable disease in nine patients (56%), and progressive disease in two patients (12%). Overall response rate (CR + PR) was 31% (95% CI, 11% to 58.7%). Four of five responders had received prior chemotherapy for KS. Over 202 cycles, grade 3 to 4 adverse events at least possibly attributed to therapy included hypertension (n = 7), neutropenia (n = 5), cellulitis (n = 3), and headache (n = 2).

Conclusion
Bevacizumab is tolerated in patients with HIV-KS and has activity in a subset of patients.

J Clin Oncol 30:1476-1483. © 2012 by American Society of Clinical Oncology

INTRODUCTION

Kaposi's sarcoma (KS) is a multifocal angioproliferative malignancy characterized by endothelial-derived spindle cells, vascular slits with enhanced permeability, and local inflammatory infiltrate. KS-associated herpes virus (KSHV), also called human herpesvirus-8, is a necessary but insufficient cause of KS.[1-3] The majority of cells in KS lesions are KSHV infected. HIV is a cofactor that increases KS risk.[4] Despite decline in KS incidence associated with highly active antiretroviral therapy (HAART) availability in the developed world, HIV-infected individuals remain at a markedly elevated risk of KS. In the United States, KS remains the second most common cancer among people with HIV.[5] KS is one of the most common cancers in sub-Saharan Africa[6] and is a major public health problem as a result of epidemic HIV.[7] KS incidence increases with age, and the effect of an aging US HIV-positive population on KS incidence remains to be seen.

HAART is essential to HIV-associated KS (HIV-KS) therapy.[8-10] Its effectiveness is largely a result of control of HIV and resulting improved KSHV-specific cellular immunity.[11] In controlled KS trials, HAART alone induced responses in approximately 20% of patients,[9,10,12,13] depending partly on immune reconstitution potential and extent of KS. Addition of systemic cytotoxic chemotherapy is indicated for advanced or symptomatic KS. Liposomal anthracyclines, with an overall response rate (ORR) of 55% to 76% in the HAART era,[13-17] are considered first-line agents. However, KS is not curable, and 1-year progression-free survival (PFS) with

liposomal doxorubicin is approximately 70%.[17] Long-term administration of continuous or intermittent chemotherapy is often required. Given substantially improved survival of HIV-positive patients on HAART, long-term toxicities of anti-KS therapies must be considered. Indeed, cumulative therapy-associated toxicity, rather than therapy-refractory disease, frequently limits long-term KS management. High cumulative doses of anthracyclines are associated with irreversible cardiac toxicity.[18] Although drugs such as interferon alfa, vincristine, vinblastine, etoposide, and paclitaxel are active in KS, they have lower activity than liposomal anthracyclines and/or greater toxicity. Improved therapies are urgently needed.[19-21]

KS, characterized by angiogenic proliferation of endothelial-derived cells, is a rational and potentially optimal tumor in which to consider antiangiogenic approaches. Vascular endothelial growth factor-A (VEGF-A) is an important paracrine and autocrine growth factor in KS.[22,23] KSHV has developed redundant mechanisms for upregulation of VEGF-A. Viral gene products, including viral G protein–coupled receptor, viral interleukin (IL) -6, latency-associated nuclear antigen (LANA), and K1, all directly or indirectly upregulate VEGF-A production.[24-29] VEGF-A seems to be responsible for leaky blood vessels, a common pathologic feature, as well as some clinical features, including tumor-associated edema and effusions.[30-33] Given the role of VEGF-A in KS pathogenesis, we performed a phase II study of the humanized, monoclonal, anti–VEGF-A antibody, bevacizumab, in patients with HIV-KS.

PATIENTS AND METHODS

Eligibility

Patients were adults with pathologically confirmed KS and at least five evaluable cutaneous lesions. HIV-positive patients must have been on HAART for at least 1 month with evidence of progressive disease (PD) or for at least 4 months without disease regression. Additional requirements included the following: Eastern Cooperative Oncology Group performance status of ≤ 2, life expectancy of at least 6 months, systolic blood pressure less than 160 mmHg, diastolic blood pressure less than 95 mmHg, urine protein less than 1+ on dipstick or less than 500 mg on 24-hour collection, absolute neutrophil count greater than 750 cells/μL, hemoglobin greater than 9 g/dL, and platelets greater than 75,000/μL. There were no CD4 count exclusion criteria. Patients with symptomatic visceral KS, concurrent malignancies not in remission for at least 1 year, or history of thromboembolic disease were excluded.

Study Design

In this single-center phase II study, patients received bevacizumab 15 mg/kg loading dose, then bevacizumab 15 mg/kg every 3 weeks starting 1 week after the loading dose. Bevacizumab was temporarily held for systolic blood pressure greater than 160 mmHg, diastolic blood pressure greater than 95 mmHg, proteinuria greater than 2+ on dipstick or 2 g in a 24-hour collection, or platelet count less than 50,000/μL. If proteinuria did not resolve to less than 2+ or 2 g/24 hours within 4 weeks, bevacizumab was discontinued. Antihypertensive therapy was initiated for systolic blood pressure greater than 160 mmHg or diastolic blood pressure greater than 95 mmHg persisting for more than 1 week or for systolic blood pressure greater than 210 mmHg or diastolic blood pressure greater than 120 mmHg at any time. HIV-positive patients with CD4 count of less than 200 cells/μL received *Pneumocystis jiroveci* prophylaxis. *Mycobacterium avium* prophylaxis was considered if CD4 count was less than 75 cells/μL. HIV-infected patients continued HAART, with adjustments made as needed according to US guidelines.[34] Bevacizumab was continued unless patients had PD requiring cytotoxic therapy, unacceptable toxicity, lack of adherence to protocol (including HAART), or patient-requested discontinuation (ie, for elective surgery). In HIV-KS, transient progression can be seen before improvement[35]; therefore, in patients with limited KS that during interval assessment was classified as PD by modified AIDS Clinical Trial Group (ACTG) criteria[36,37] (Appendix, online only), bevacizumab could be continued for additional cycles at investigator discretion as long as the patient did not require cytotoxic chemotherapy. Filgrastim was used as clinically indicated. Bevacizumab was provided by the Cancer Therapy Evaluation Program of the National Cancer Institute (NCI) through a Cooperative Research and Development Agreement with Genentech (South San Francisco, CA). This protocol was approved by the NCI Institutional Review Board and is

Table 1. Demographics and Clinical Characteristics of 17 Patients With HIV-KS

Demographic or Clinical Characteristic	No. of Patients	%
Age, years		
Median	44	
Range	23-65	
Sex		
Male	16	94
Female	1	6
Race		
Black	8	47
White	6	35
Hispanic	3	18
KS prognostic factors*		
T_1	13	76
I_1	5	29
S_1	4	24
Revised TS staging†		
Good (T_0S_0, T_1S_0, or T_0S_1)	2	12
Poor (T_1S_1)	15	88
CD4 count, cells/μL		
Median	294	
Range	7-654	
< 200	7	41
Time on HAART, months		
Median	12	
Range	1-90	
HIV viral load, copies/mL		
Median	< 50	
Range	< 50-180,000	
< 50	14	82
Detectable circulating KSHV‡	5	31
Prior therapy for KS		
Chemotherapy	13	76
Liposomal doxorubicin	12	71
Paclitaxel	5	29
Other§	2	12
Immunotherapy	11	65
Radiation	5	29
Time since last chemotherapy		
Median	3 months	
Range	3 weeks-8 years	

Abbreviations: HAART, highly active antiretroviral therapy; KS, Kaposi's sarcoma; KSHV, Kaposi's sarcoma–associated herpes virus; TS, staging based on tumor and systemic illness.

*Risk factors based on AIDS Clinical Trial Group staging criteria for extent of tumor (T), immune status (I), and systemic illness (S), as follows: T_1, edema or ulceration, extensive oral mucosa KS, or visceral KS; I_1, CD4 < 150 cells/μL; and S_1, history of opportunistic infections or thrush, and/or "B" symptoms present, and/or Karnofsky score < 70, and/or other HIV-related disease.

†Revised AIDS KS prognostic criteria exclude CD4 as risk factor.

‡Baseline peripheral-blood mononuclear cell–associated KSHV viral load[38] was assessed for the 16 evaluable patients. The five patients with detectable KSHV had a median of 190 copies/10^6 cells (range, 17 to 3,200 copies/10^6 cells).

§Other chemotherapies included etoposide, vincristine, vinblastine, vinorelbine, and bleomycin.

registered at ClinicalTrials.gov (NCT00055237). All patients provided written informed consent.

Efficacy and Safety Assessments

KS response was evaluated every cycle and categorized as complete response (CR), partial response (PR), stable disease (SD), or PD using modified ACTG criteria,[36,37] as previously described.[37] Response evaluations included lesion counts, measurement of the sum product of the diameters (SPD) of five marker lesions, and assessment of nodularity. PR required at least 50% decrease in number of lesions and/or sum product of the diameters of marker lesions and/or nodularity of lesions and no new lesions. CR required clinical resolution of all lesions and tumor-associated phenomenon, with biopsy confirmation. (See Appendix for detailed response criteria.) Both CR and PR had to be sustained for 4 weeks. Best response was evaluated for each patient. Patients who did not achieve SD for at least 3 weeks were considered to have PD as best response.

Safety was monitored each cycle and 3 to 6 weeks after completing therapy. Evaluations included CBCs with differential, serum chemistries, and urinalysis. Toxicities were graded using NCI Common Terminology Criteria for Adverse Events version 2.0. In HIV-positive patients, CD4 cell counts and HIV viral load were evaluated every 12 weeks.

Correlative Assays

Correlative assays were performed on stored biospecimens collected at baseline and time of best response. Serum VEGF-A was measured using Quantikine ELISA Kit (R&D Systems, Minneapolis, MN). Serum cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12 p70, interferon gamma, tumor necrosis factor α) were evaluated using MSD 96-Well Multiarray Proinflammatory 7-plex Assay (Meso-Scale Discovery, Gaithersburg, MD) and Sector Imager (Meso-Scale Discovery). KSHV viral load was measured using previous described methodology.[38]

Statistical Considerations

The primary objective was to determine the ORR (CR + PR) in patients with HIV-KS on HAART treated with bevacizumab. HIV-negative patients were also eligible; however, given likely differences between HIV-negative KS and HIV-KS, prespecified primary analysis was limited to HIV-KS. Entry criteria requiring SD or PD on HAART were designed to exclude patients most likely to respond to HAART alone. Sample size was determined using two-stage Simon optimal design[39] ($\alpha = .10$; $\beta = .10$; undesirably low ORR, 15%; targeted ORR, 45%). If two or more of the first eight patients had a PR or better, accrual continued to 17 HIV-positive patients. If five or more of 17 patients responded, bevacizumab would be considered sufficiently active to

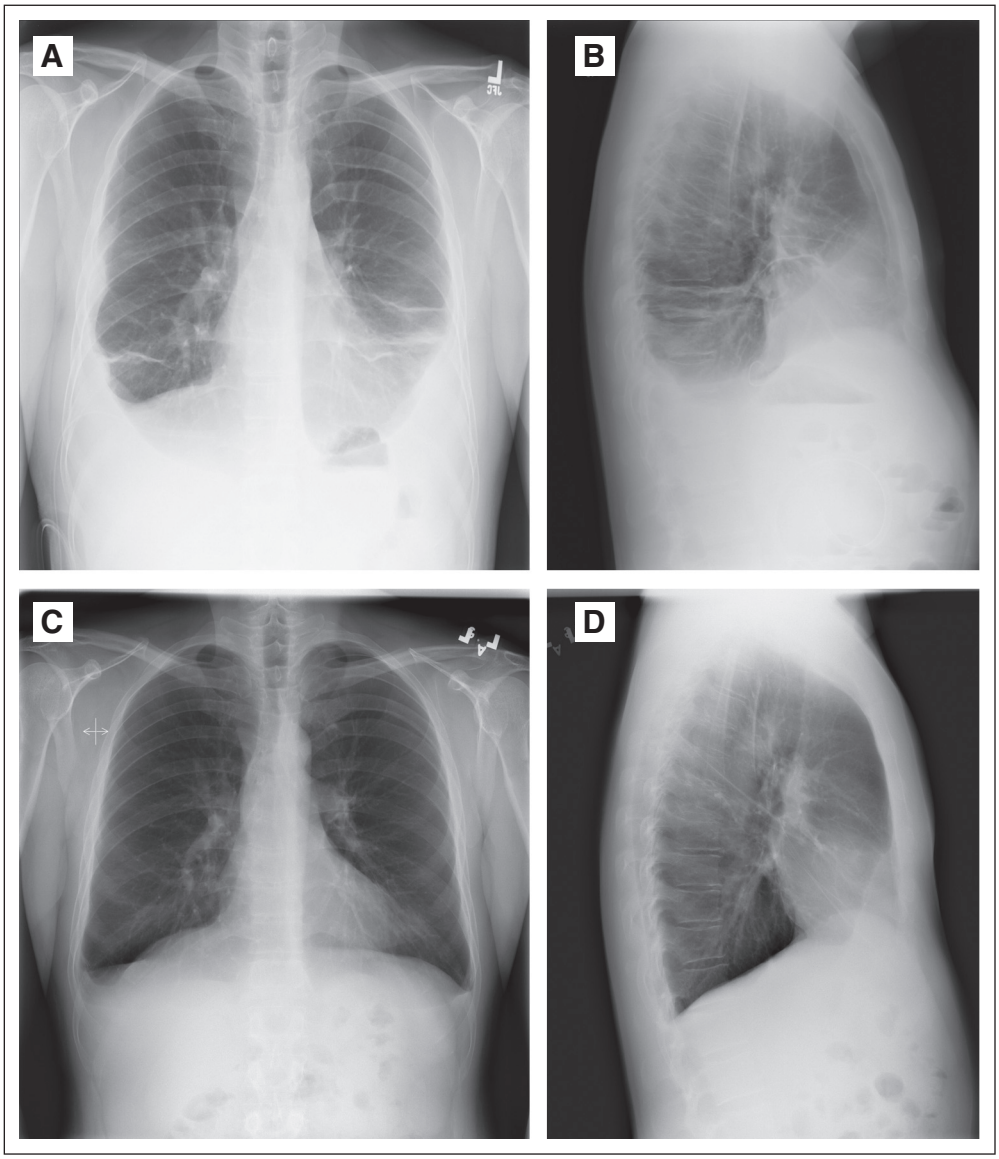

Fig 1. Resolution of a Kaposi's sarcoma (KS) –associated chylous pleural effusion in a patient receiving bevacizumab. A 44-year-old man with HIV and KS had dramatic worsening of KS after starting highly active antiretroviral therapy, with development of greater than 50 cutaneous lesions, KS involving pelvic and inguinal lymph nodes, bilateral pleural effusions draining up to 6 L a week, and bilateral lower extremity edema. Analysis of the effusions did not reveal evidence of primary effusion lymphoma. The patient received 10 cycles of liposomal doxorubicin with some improvement in cutaneous lesions but no resolution of effusion, which required a right-sided indwelling pleural catheter. He also had continued lower extremity edema requiring daily diuretics. Within the first two cycles of bevacizumab, the effusions stopped draining, lower extremity swelling resolved, and diuretics were discontinued. (A) Posterior-anterior and (B) lateral chest x-rays at baseline. Bilateral pleural effusions and indwelling pleural catheter are on right. Pleural fluid was chylous (effusion triglycerides 1,905 mg/dL). (C) Posterior-anterior and (D) lateral chest x-rays at month 3 on bevacizumab. The effusions are resolved, and the indwelling catheter has been removed.

consider future studies. Statistical significance of differences in serum VEGF and cytokines at time of best response versus baseline was determined by Wilcoxon signed rank test. Significance of the difference in changes between patients with or without a clinical response was determined by an exact Wilcoxon rank sum test. PFS for evaluable patients with HIV was determined using Kaplan-Meier methods, censoring patients without progression at the off-study date. *P* values are two-tailed and presented without adjustment for multiple comparisons, because they are results of exploratory tests.

RESULTS

Patient Characteristics

Between February 2003 and December 2008, 17 patients (16 men and one woman) with HIV-KS were enrolled (Table 1). Eight patients were black, six were white, and three were Hispanic. Median age was 44 years (range, 23 to 65 years). Thirteen patients (76%) had advanced KS (ACTG T_1).[40] Median CD4 count was 294 cells/μL (range, 7 to 654 cells/μL), and five patients (29%) had CD4 count less than 150 cells/μL. Patients had substantial prior treatment; 13 patients (76%) had received previous cytotoxic chemotherapy, including liposomal doxorubicin (n = 12) and paclitaxel (n = 5). Additionally, 11 patients had received immunotherapy (interferon alfa, IL-12, or thalidomide), and five patients had received radiation therapy (Table 1). One patient had bilateral KS-associated pleural effusions requiring indwelling catheter drainage (Fig 1) and bilateral lower extremity edema requiring daily diuretics. Additionally, two HIV-uninfected black men, age 49 and 65, were enrolled and analyzed separately.

Treatment

Assessable patients with HIV-KS received a median of 11 cycles (range, four to 37 cycles) of bevacizumab and were observed on-study for a median of 9 months (range, 3 to 36 months). All patients with HIV-KS received HAART. The two HIV-uninfected patients received four and five cycles of bevacizumab. Two hundred two cycles are evaluable for safety and tolerability.

Efficacy

Sixteen of 17 patients with HIV-KS were assessable for tumor response; one patient did not return for tumor evaluation after initial doses of therapy. Best responses were CR in three patients (19%), PR in two patients (12%), SD in nine patients (56%), and PD in two patients (12%; Table 2). Best ORR was 31% (95% CI, 11% to 58.7%). One patient achieved CR after initial transient progression during the first month. Another patient had rapid durable resolution of bilateral effusions and lower extremity edema and achieved a CR (Fig 1). In patients with PR or CR, median time to best response was 5 months (range, 2.5 to 9.5 months). In two HIV-uninfected patients, best responses were SD (n = 1) and PD (n = 1). Overall, eight of 11 patients with baseline tumor-associated edema had evidence of improvement. Six patients had a greater than 2 cm decrease in circumference of affected limb at time of best response, and five patients had subjective improvement, including opiate discontinuation (n = 2), diuretic discontinuation (n = 1), and increased mobility and activity (n = 4).

All five responders were among a group of 12 patients with sustained HIV suppression on study (Table 2). In these 12 patients, ORR was 42%. Interestingly, 11 patients had evidence of ongoing immune reconstitution, with median change in CD4 count of +144 cells/μL (range, −51 to +352 cells/μL). Seven (42%) of these 12 patients had been on cytotoxic chemotherapy in the 12 months before starting bevacizumab, and immune reconstitution may have been facilitated by switching from additional cytotoxic therapy to bevacizumab. Responses did not seem to be merely a result of recent initiation of HAART, because all responders had been on a stable HAART regimen for 6 months or longer (median, 11 months) before starting bevacizumab. In contrast, four patients had increasing HIV viral load on study attributed to HAART nonadherence (n = 2), acquired HIV resistance (n = 1), or not taking HAART because of intercurrent illness (n = 1). In these four patients, median change in CD4 count was −19 cells/μL (range, −232 to +37 cells/μL; Table 2). In evaluable

Table 2. Response, HIV Control, CD4 Dynamics, TS Prognosis, and History of Therapy for KS

Response	No. of Patients	Time on HAART* at Baseline for Each Patient (months)	Baseline HIV Viral Load (copies/mL)		CD4 Change (cells/μL)		TS† Poor Prognosis (No. of patients)	Previous Cytotoxic Chemotherapy for KS (No. of Patients)
			Median	Range	Median	Range		
HIV well controlled on study‡								
CR	3	8, 11, 12	< 50	< 50-145§	198	85-270	3	2‖
PR	2	6, 50	< 50	< 50-< 50	187	21-352	2	2‖
SD	6	3, 3, 21, 26, 45, 90	< 50	< 50-< 50	96	−51-243	4	4
PD	1	6	< 50		149		1	1
HIV not consistently controlled on study¶								
SD	3	12, 15, 51	< 50	< 50-180,000	−35	−2-−237	3	3
PD	1	9	180		37		1	0

Abbreviations: CR, complete response; HAART, highly active antiretroviral therapy; KS, Kaposi's sarcoma; PD, progressive disease; PR, partial response; SD, stable disease; TS, staging based on tumor and systemic illness.

*Months on the specific HAART regimen used at time of the screening visit.

†Revised AIDS KS prognostic criteria, excludes CD4 as prognostic factor.[41]

‡HIV is considered well controlled if viral load is < 200 copies/mL while on study (median, < 50 copies/mL; range, < 50 to 102 copies/mL).

§Patient with HIV viral load of 145 copies/mL had been adherent to HAART for 12 months and had an HIV viral load < 50 copies/mL 2 months prior. Low-level HIV viremia at the baseline visit was attributed to use of a different polymerase chain reaction assay.[42]

‖Four of five responding patients had been previously treated with cytotoxic chemotherapy. All four patients had received prior liposomal doxorubicin (median cumulative dose, 260 mg/m^2; range, 120 to 600 mg/m^2). In addition, one patient received prior bleomycin and vincristine, and two patients had prior radiation therapy.

¶Median HIV viral load when measured at off-study visit was 110,000 copies/mL (range, 16,400 to 182,000 copies/mL).

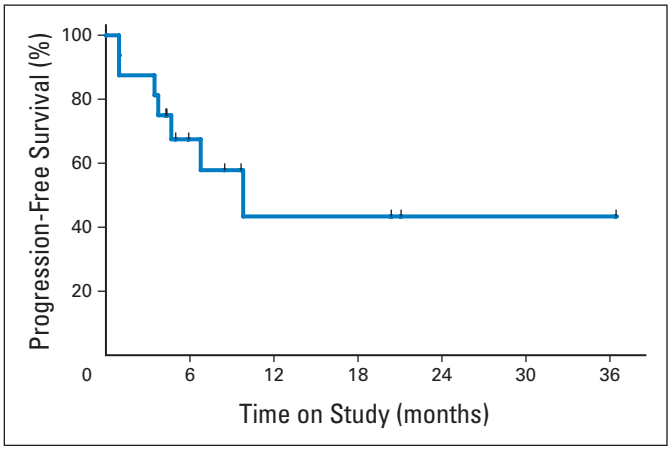

Fig 2. Kaplan-Meier progression-free survival (PFS) curve in 16 patients with HIV-associated Kaposi's sarcoma (KS) on highly active antiretroviral therapy treated with bevacizumab. Median follow-up until progression or censoring for these 16 patients was 5.4 months (range, 3 to 36 months). Median time to progression was 8.3 months. Hatch marks denote time patients are censored. One patient with limited KS who had transient initial progression followed by lasting complete response was considered progression free in PFS analysis.

patients with HIV-KS, median PFS was 8.3 months (Fig 2). Overall survival on study was 100%.

Toxicities

Bevacizumab was generally well tolerated. Common adverse effects were proteinuria, headache, epistaxis, and hypertension (Table 3). Five patients started antihypertensive therapy. One heavily pretreated patient with lasting PR developed asymptomatic proteinuria after cycle 30 and discontinued bevacizumab after 37 cycles when urine protein reached 1,024 mg/24 h. Proteinuria resolved over 7.5 months of follow-up. A second patient with CR who discontinued therapy after cycle 26 was found to have an enlarged cardiac silhouette on chest x-ray 3 months after completing bevacizumab. On echocardiography, ejection fraction (EF) was 36%; no baseline cardiac studies were available for comparison. The patient was asymptomatic and continued on losartan and carvedilol. Follow-up echocardiography 6 years later showed an EF of 50% with no dilation. Three patients developed soft tissue infections associated with underlying KS requiring intravenous antibiotics; one patient had recurrent infections, one had infection in the setting of neutropenia, and one discontinued therapy as a result of severity of infection.

Changes in Serum VEGF and Cytokines

We assessed serum levels of VEGF-A and several cytokines possibly relevant to KS pathogenesis at entry and time of best response (Table 4). There was a decrease in serum IL-8 (median decrease, 11.2 ng/mL; $P = .0023$) but no significant changes in serum VEGF-A, IL-1β, IL-6, IL-8, IL-10, IL-12p70, interferon gamma, or tumor necrosis factor α. There were no significant differences in changes in these factors between those with or without a clinical response.

Table 3. Select Adverse Events Possibly, Probably, or Definitely Attributed to Bevacizumab Over 202 Cycles in 19 Patients

	Grade 1		Grade 2		Grade 3		Grade 4	
Toxicity	No.	%	No.	%	No.	%	No.	%
Proteinuria								
Events	32	16	12	6				
Patients	12	63	3	16				
Hypertension*								
Events	12	6			7	3		
Patients	1	5			6	32		
LV dysfunction								
Events			1	< 1				
Patients			1	5				
Thrombosis†								
Events			1	< 1				
Patients			1	5				
Neutropenia‡								
Events	3	1	8	2	5	1		
Patients			3	16	2	11		
Anemia								
Events	1	< 1						
Patients	1	5						
Thrombocytopenia								
Events	10	5						
Patients	5	26						
Epistaxis								
Events	11	5						
Patients	8	42						
Infection§								
Events	4	2	5	2	2	1	1	< 1
Patients	2	11	2	11	2	11	1	5
Headache								
Events	12	6	3	1	2	1		
Patients	3	16	3	16	2	11		
Vomit								
Events	1	< 1			1	< 1		
Patients	1	5			1	5		

NOTE. All 19 patients are included in the toxicity evaluation. The median number of cycles is nine (range, one to 37 cycles). Number of events includes adverse events over 202 cycles. Number of patients includes worst grade for each adverse event per patient over 19 patients.

Abbreviation: LV, left ventricular.

*Grade 3 hypertension was defined as addition of antihypertensive agent, with management of hypertension based on protocol guidelines. Five patients initiated antihypertensive therapy, and two of these patients needed a second antihypertensive agent added later in the protocol.

†Basilic vein intravenous line–associated thrombosis managed by pulling line.

‡Four of five instances of grade 3 neutropenia occurred in a patient with benign ethnic neutropenia[43] whose baseline absolute neutrophil count would be categorized by Common Terminology Criteria for Adverse Events 0 as a grade 2 adverse event (absolute neutrophil count, 1,000 to 1,500 cells/μL).

§Infections included soft tissue infection (n = 5), gingival infections (n = 4), mild upper respiratory tract infections (n = 2), or oral herpes (n = 1). Soft tissue infections were related to underlying Kaposi's sarcoma or poor dentition.

DISCUSSION

In this study, patients with HIV-KS on a stable HAART regimen received bevacizumab 15 mg/kg every 3 weeks after an initial loading dose. Best ORR was 31% (95% CI, 11% to 58.7%), meeting predefined criteria for consideration in future combination studies. Median PFS was 8.3 months. Compared with bevacizumab monotherapy studies in metastatic renal cell cancer or recurrent ovarian cancer, in which ORRs of 10% to 21% were seen,[44-46] responses observed in HIV-KS are quite good. Moreover, these responses were seen in patients with poor prognosis KS,[41] and four of five responding patients had received

Table 4. Evaluation of Changes in VEGF and Inflammatory Cytokines As Biomarkers of Bevacizumab Activity and Predictors of Clinical Response

	Baseline (ng/mL)		Change From Baseline to Best Clinical Response (ng/mL)			Change in Responders (ng/mL)		Change in Nonresponders (ng/mL)		
Biomarker	Median	IQR	Median	IQR	*P**	Median	IQR	Median	IQR	*P*†
VEGF	429	282-881	186	396-−286	.42	290	396-134	21.8	325-−286	.63
IL-1b	0.35	0.1-0.7	0	0-−0.2	.37	0.1	0.8-0	−0.1	0-−0.2	.14
IL-6	1.6	0.9-2.8	0	0.4-−1.8	.34	0.5	0.6-0.4	−0.4	0.1-−1.8	.11
IL-8	44.9	22-117	−11.2	−2.1-−68.5	.0023	−43	−16.4-−44.2	−9	0.2-−68.5	.29
IL-10	4.6	2.6-8.7	−0.3	1.1-−3.4	.40	0.3	2.8-−3.3	−0.8	0.3-−3.4	.58
IL-12p70	0.5	0.3-0.8	0	0.6-−0.1	.35	0.6	2-−0.2	0	0.1-0	.68
IFN-γ	1	0.5-1.9	0.3	2.9-−0.4	.13	2.9	4.7-0	0.2-	1-−0.4	.52
TNF-α	10.4	7.3-15.6	−1.8	0.9-−6	.13	−3	0.2-−5.7	−0.7	1.2-−6	.63

Abbreviations: IFN, interferon; IL, interleukin; IQR, interquartile range; VEGF, vascular endothelial growth factor.
*Nonparametric analysis of change in biomarker (baseline–best clinical response) performed using Wilcoxon signed rank test.
†Association of change in biomarker (baseline–best clinical response) according to those with or without a major clinical response performed using an exact Wilcoxon rank sum test.

prior cytotoxic chemotherapy (Table 2). In contrast to most cytotoxic agents active in KS, bevacizumab does not seem to impair immune reconstitution, an important feature for therapeutic interventions for HIV-KS.

Patients who responded had controlled HIV and increases in CD4 counts while on bevacizumab (Table 2). Baseline CD4 lymphocytopenia may have been in part a result of prior chemotherapy in most patients, and cessation of hematotoxic chemotherapy seems to permit the increases in CD4 counts observed in responding patients. A limitation to any phase II study in HIV-KS is that possible immune reconstitution must be taken into consideration, and HAART alone can induce responses in KS (approximately 20% in controlled trials[9,13]). Meta-analysis suggests that most patients with HIV-KS who respond to HAART alone have T_0 KS (limited disease); only five documented cases were identified in which patients with T_1 KS (widespread disease) responded to HAART alone.[10] In the current study, all responders had T_1 KS, and most had prior cytotoxic chemotherapy, making it unlikely that responses were a result of HAART alone. Moreover, most KS responses to HAART occur soon after the initiation of HAART, although paradoxical worsening of HIV-KS after starting HAART is also described.[47,48] To limit these potential biases, we required patients to have either KS not regressing while on HAART for 4 months or progressing on HAART for 1 month. Given the potential role of VEGF dysregulation in the pathophysiology of KS-associated pleural effusions[49] and edema, rapid resolution of pleural effusions in one patient and common subjective improvement in tumor-associated edema were noteworthy observations. Nonetheless, definitive assessment of anti-KS efficacy of bevacizumab beyond that of HAART alone requires a randomized controlled trial.

With the exception of decrease from baseline in IL-8, assessment of serum VEGF-A and cytokines did not show substantial changes or association with responses. Bevacizumab binds to VEGF-A, and measurement of bound VEGF-A may affect assay results.[50] Although difference between responders and nonresponders was not statistically significant, the decrease in IL-8 is interesting, because KSHV encodes a latently expressed gene, *K13*, that transcriptionally upregulates IL-8[51] and may have a role in mediating angiogenesis in KS.[52] Additional studies will be needed to evaluate IL-8 as a biomarker and to sort out its possible biologic role in KS response to bevacizumab.

Bevacizumab was generally well tolerated over a relatively long time. Adverse events (Table 3) were comparable with those seen in other studies.[53] Two noteworthy toxicities at least possibly attributable to long-term bevacizumab were proteinuria ($>$ 1 g/d) in one patient and a decreased cardiac EF in another; in both cases, toxicities improved with bevacizumab discontinuation. In addition, five patients required initiation of antihypertensive agents. Three patients developed soft tissue infections; KS patients are susceptible to soft tissue infections, and it was unclear whether bevacizumab had a role in their pathogenesis. Overall, the toxicity profile observed in this HIV-positive population receiving HAART supports bevacizumab use in future studies in HIV-associated cancers, as well as its use in HIV-positive patients with cancers for which bevacizumab is US Food and Drug Administration approved. This is particularly important given the increasing burden of non–AIDS-defining malignancies such as lung cancer and colon cancer in HIV-infected individuals in the United States.[5]

KS response rates are affected by extent of disease, degree of immunosuppression, and control of HIV, and response rates can be difficult to compare among clinical trials. The observed ORR here is less than that reported with liposomal anthracyclines[13-16] but is comparable to that seen using agents that inhibit angiogenesis through different mechanisms, such as TNP-470[54] or the matrix metalloproteinase inhibitor COL-3.[55,56] Given the important role of VEGF-A in KS pathogenesis, one must ask why bevacizumab was not more active. One likely reason is that redundant angiogenic and proliferative stimuli activate spindle cell proliferation. In addition to VEGF-A receptors 1 and 2, KS spindle cells express VEGF-A receptor 3 and the receptor for platelet-derived growth factor (PDGF) and proliferate in response to ligands for these receptors (VEGF-C and PDGF).[57-60] Furthermore, a number of KSHV genes, such as latency-associated nuclear antigen (*LANA*), *v-FLIP*, *v-cyclin*, and *kaposin-A*, can inhibit apoptosis or directly contribute to KS spindle cell proliferation.[61] Thus, optimal targeted therapy for KS may require targeting two or more of these pathways simultaneously.[54-56]

Although only a subset of patients responded in this trial, results should be considered in light of the fact that most patients had features making them unlikely to respond to any therapy. Overall, this study suggests that bevacizumab has utility in KS. In particular, bevacizumab

may be of value in combination with other drugs or after initial reduction of the tumor burden with cytotoxic chemotherapy, or in patients who are approaching the maximal safe cumulative dose of anthracyclines. A second study of bevacizumab combined with liposomal doxorubicin, followed by bevacizumab maintenance (ClinicalTrials.gov identifier: NCT00923936), is under way, and a randomized trial of bevacizumab is worth considering. With increasing insight into KSHV biology and range of clinical presentations of KS[38] and other KSHV-associated malignancies, rational therapeutic approaches such as bevacizumab offer hope for both cytotoxic-sparing treatment options and personalized approaches to difficult-to-manage specific tumor-associated symptoms like chronic edema and effusions.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** None **Stock Ownership:** None **Honoraria:** None **Research Funding:** Robert Yarchoan, bevacizumab provided to National Cancer Institute by Genentech under a Cooperative Research and Development Agreement **Expert Testimony:** None **Other Remuneration:** Robert Yarchoan, patents on certain anti-HIV drugs, including didanosine, assigned to Department of Health and Human Services/National Institutes of Health under the Federal Technology Transfer Act

AUTHOR CONTRIBUTIONS

Conception and design: Richard F. Little, Robert Yarchoan
Collection and assembly of data: Thomas S. Uldrick, Kathleen M. Wyvill, Pallavi Kumar, Deirdre O'Mahony, Wendy Bernstein, Karen Aleman, Mark N. Polizzotto, Stefania Pittaluga, Vickie Marshall, Denise Whitby, Richard F. Little, Robert Yarchoan
Data analysis and interpretation: Thomas S. Uldrick, Kathleen M. Wyvill, Deirdre O'Mahony, Mark N. Polizzotto, Seth M. Steinberg, Robert Yarchoan
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Chang Y, Cesarman E, Pessin MS, et al: Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266: 1865-1869, 1994

2. Whitby D, Howard MR, Tenant-Flowers M, et al: Detection of Kaposi sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. Lancet 346: 799-802, 1995

3. Moore PS, Kingsley LA, Holmberg SD, et al: Kaposi's sarcoma-associated herpesvirus infection prior to onset of Kaposi's sarcoma. AIDS 10:175-180, 1996

4. Engels EA, Biggar RJ, Hall HI, et al: Cancer risk in people infected with human immunodeficiency virus in the United States. Int J Cancer 123:187-194, 2008

5. Shiels MS, Pfeiffer RM, Gail MH, et al: Cancer burden in the HIV-infected population in the United States. J Natl Cancer Inst 103:753-762, 2011

6. Chokunonga E, Levy LM, Bassett MT, et al: AIDS and cancer in Africa: The evolving epidemic in Zimbabwe. AIDS 13:2583-2588, 1999

7. Mosam A, Carrara H, Shaik F, et al: Increasing incidence of Kaposi's sarcoma in black South Africans in KwaZulu-Natal, South Africa (1983-2006). Int J STD AIDS 20:553-556, 2009

8. Dupont C, Vasseur E, Beauchet A, et al: Long-term efficacy on Kaposi's sarcoma of highly active antiretroviral therapy in a cohort of HIV-positive patients: CISIH 92—Centre d'Information et de Soins de l'Immunodeficience Humaine. AIDS 14:987-993, 2000

9. Noy A, Scadden DT, Lee J, et al: Angiogenesis inhibitor IM862 is ineffective against AIDS-Kaposi's sarcoma in a phase III trial, but demonstrates sustained, potent effect of highly active antiretroviral therapy: From the AIDS Malignancy Consortium and IM862 Study Team. J Clin Oncol 23:990-998, 2005

10. Krown SE: Highly active antiretroviral therapy in AIDS-associated Kaposi's sarcoma: Implications for the design of therapeutic trials in patients with advanced, symptomatic Kaposi's sarcoma. J Clin Oncol 22:399-402, 2004

11. Guihot A, Dupin N, Marcelin AG, et al: Low T cell responses to human herpesvirus 8 in patients with AIDS-related and classic Kaposi sarcoma. J Infect Dis 194:1078-1088, 2006

12. Tulpule A, Scadden DT, Espina BM, et al: Results of a randomized study of IM862 nasal solution in the treatment of AIDS-related Kaposi's sarcoma. J Clin Oncol 18:716-723, 2000

13. Martin-Carbonero L, Barrios A, Saballs P, et al: Pegylated liposomal doxorubicin plus highly active antiretroviral therapy versus highly active antiretroviral therapy alone in HIV patients with Kaposi's sarcoma. AIDS 18:1737-1740, 2004

14. Northfelt DW, Dezube BJ, Thommes JA, et al: Pegylated-liposomal doxorubicin versus doxorubicin, bleomycin, and vincristine in the treatment of AIDS-related Kaposi's sarcoma: Results of a randomized phase III clinical trial. J Clin Oncol 16:2445-2451, 1998

15. Stewart S, Jablonowski H, Goebel FD, et al: Randomized comparative trial of pegylated liposomal doxorubicin versus bleomycin and vincristine in the treatment of AIDS-related Kaposi's sarcoma: International Pegylated Liposomal Doxorubicin Study Group. J Clin Oncol 16:683-691, 1998

16. Cooley T, Henry D, Tonda M, et al: A randomized, double-blind study of pegylated liposomal doxorubicin for the treatment of AIDS-related Kaposi's sarcoma. Oncologist 12:114-123, 2007

17. Martín-Carbonero L, Palacios R, Valencia E, et al: Long-term prognosis of HIV-infected patients with Kaposi sarcoma treated with pegylated liposomal doxorubicin. Clin Infect Dis 47:410-417, 2008

18. Smith LA, Cornelius VR, Plummer CJ, et al: Cardiotoxicity of anthracycline agents for the treatment of cancer: Systematic review and meta-analysis of randomised controlled trials. BMC Cancer 10:337, 2010

19. Yarchoan R, Tosato G, Little RF: Therapy insight: AIDS-related malignancies: The influence of antiviral therapy on pathogenesis and management. Nat Clin Pract Oncol 2:406-415, 2005

20. Di Lorenzo G, Konstantinopoulos PA, Pantanowitz L, et al: Management of AIDS-related Kaposi's sarcoma. Lancet Oncol 8:167-176,2007

21. Uldrick TS, Whitby D: Update on KSHV epidemiology, Kaposi sarcoma pathogenesis, and treatment of Kaposi sarcoma. Cancer Lett 305:150-162, 2011

22. Brown LF, Tognazzi K, Dvorak HF, et al: Strong expression of kinase insert domain-containing receptor, a vascular permeability factor/vascular endothelial growth factor receptor in AIDS-associated Kaposi's sarcoma and cutaneous angiosarcoma. Am J Pathol 148:1065-1074, 1996

23. Masood R, Cai J, Zheng T, et al: Vascular endothelial growth factor/vascular permeability factor is an autocrine growth factor for AIDS-Kaposi sarcoma. Proc Natl Acad Sci U S A 94:979-984, 1997

24. Bais C, Santomasso B, Coso O, et al: G-protein-coupled receptor of Kaposi's sarcoma-associated herpesvirus is a viral oncogene and angiogenesis activator. Nature 391:86-89, 1998

25. Cannon M, Philpott NJ, Cesarman E: The Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor has broad signaling effects in primary effusion lymphoma cells. J Virol 77:57-67, 2003

26. Aoki Y, Tosato G: Role of vascular endothelial growth factor/vascular permeability factor in the pathogenesis of Kaposi's sarcoma-associated herpesvirus-infected primary effusion lymphomas. Blood 94:4247-4254, 1999

27. Cai Q, Murakami M, Si H, et al: A potential alpha-helix motif in the amino terminus of LANA encoded by Kaposi's sarcoma-associated herpesvirus is critical for nuclear accumulation of HIF-1alpha in normoxia. J Virol 81:10413-10423, 2007

28. Prakash O, Swamy OR, Peng X, et al: Activation of Src kinase Lyn by the Kaposi sarcoma-associated herpesvirus K1 protein: Implications for lymphomagenesis. Blood 105:3987-3994, 2005

29. Wang L, Wakisaka N, Tomlinson CC, et al: The Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) K1 protein induces expression of angiogenic and invasion factors. Cancer Res 64:2774-2781, 2004

30. Aoki Y, Tosato G, Nambu Y, et al: Detection of vascular endothelial growth factor in AIDS-related primary effusion lymphomas. Blood 95:1109-1110, 2000

31. Nagy JA, Masse EM, Herzberg KT, et al: Pathogenesis of ascites tumor growth: Vascular permeability factor, vascular hyperpermeability, and ascites fluid accumulation. Cancer Res 55:360-368, 1995

32. Nagy JA, Morgan ES, Herzberg KT, et al: Pathogenesis of ascites tumor growth: Angiogenesis, vascular remodeling, and stroma formation in the peritoneal lining. Cancer Res 55:376-385, 1995

33. Haddad L, El Hajj H, Abou-Merhi R, et al: KSHV-transformed primary effusion lymphoma cells induce a VEGF-dependent angiogenesis and establish functional gap junctions with endothelial cells. Leukemia 22:826-834, 2008

34. Panel on Antiretroviral Guidelines for Adults and Adolescents: Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. Washington, DC, Department of Health and Human Services, 2009, pp 1-161

35. Little RF, Pluda JM, Wyvill KM, et al: Activity of subcutaneous interleukin-12 in AIDS-related Kaposi sarcoma. Blood 107:4650-4657, 2006

36. Krown SE, Metroka C, Wernz JC: Kaposi's sarcoma in the acquired immune deficiency syndrome: A proposal for uniform evaluation, response, and staging criteria—AIDS Clinical Trials Group Oncology Committee. J Clin Oncol 7:1201-1207, 1989

37. Welles L, Saville MW, Lietzau J, et al: Phase II trial with dose titration of paclitaxel for the therapy of human immunodeficiency virus-associated Kaposi's sarcoma. J Clin Oncol 16:1112-1121, 1998

38. Uldrick TS, Wang V, O'Mahony D, et al: An interleukin-6-related systemic inflammatory syndrome in patients co-infected with Kaposi sarcoma-associated herpesvirus and HIV but without multicentric Castleman disease. Clin Infect Dis 51:350-358, 2010

39. Simon R: Optimal two-stage designs for phase II clinical trials. Control Clin Trials 10:1-10, 1989

40. Krown SE, Testa MA, Huang J: AIDS-related Kaposi's sarcoma: Prospective validation of the AIDS Clinical Trials Group staging classification—AIDS Clinical Trials Group Oncology Committee. J Clin Oncol 15:3085-3092, 1997

41. Nasti G, Talamini R, Antinori A, et al: AIDS-related Kaposi's sarcoma: Evaluation of potential new prognostic factors and assessment of the AIDS Clinical Trial Group Staging System in the HAART era—The Italian Cooperative Group on AIDS and Tumors and the Italian Cohort of Patients Naive From Antiretrovirals. J Clin Oncol 21:2876-2882, 2003

42. Do T, Duncan J, Butcher A, et al: Comparative frequencies of HIV low-level viremia between real-time viral load assays at clinically relevant thresholds. J Clin Virol 52:S83-S89, 2011 (suppl 1)

43. Hsieh MM, Tisdale JF, Rodgers GP, et al: Neutrophil count in African Americans: Lowering the target cutoff to initiate or resume chemotherapy? J Clin Oncol 28:1633-1637, 2010

44. Yang JC, Haworth L, Sherry RM, et al: A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med 349:427-434, 2003

45. Cannistra SA, Matulonis UA, Penson RT, et al: Phase II study of bevacizumab in patients with platinum-resistant ovarian cancer or peritoneal serous cancer. J Clin Oncol 25:5180-5186, 2007

46. Burger RA, Sill MW, Monk BJ, et al: Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: A Gynecologic Oncology Group study. J Clin Oncol 25:5165-5171, 2007

47. Bower M, Nelson M, Young AM, et al: Immune reconstitution inflammatory syndrome associated with Kaposi's sarcoma. J Clin Oncol 23:5224-5228, 2005

48. Leidner RS, Aboulafia DM: Recrudescent Kaposi's sarcoma after initiation of HAART: A manifestation of immune reconstitution syndrome. AIDS Patient Care STDS 19:635-644, 2005

49. Grove CS, Lee YC: Vascular endothelial growth factor: The key mediator in pleural effusion formation. Curr Opin Pulm Med 8:294-301, 2002

50. Jain RK, Duda DG, Willett CG, et al: Biomarkers of response and resistance to antiangiogenic therapy. Nat Rev Clin Oncol 6:327-338, 2009

51. Sun Q,Matta H, Lu G, et al: Induction of IL-8 expression by human herpesvirus 8 encoded vFLIP K13 via NF-kappa B activation. Oncogene 25:2717-2726, 2006

52. Lane BR, Liu J, Bock PJ, et al: Interleukin-8 and growth-regulated oncogene alpha mediate angiogenesis in Kaposi's sarcoma. J Virol 76:11570-11583, 2002

53. Gressett SM, Shah SR: Intricacies of bevacizumab-induced toxicities and their management. Ann Pharmacother 43:490-501, 2009

54. Dezube BJ, Von Roenn JH, Holden-Wiltse J, et al: Fumagillin analog in the treatment of Kaposi's sarcoma: A phase I AIDS Clinical Trial Group study—AIDS Clinical Trial Group No. 215 Team. J Clin Oncol 16:1444-1449, 1998

55. Cianfrocca M, Cooley TP, Lee JY, et al: Matrix metalloproteinase inhibitor COL-3 in the treatment of AIDS-related Kaposi's sarcoma: A phase I AIDS malignancy consortium study. J Clin Oncol 20:153-159, 2002

56. Dezube BJ, Krown SE, Lee JY, et al: Randomized phase II trial of matrix metalloproteinase inhibitor COL-3 in AIDS-related Kaposi's sarcoma: An AIDS Malignancy Consortium Study. J Clin Oncol 24:1389-1394, 2006

57. Folpe AL, Veikkola T, Valtola R, et al: Vascular endothelial growth factor receptor-3 (VEGFR-3): A marker of vascular tumors with presumed lymphatic differentiation, including Kaposi's sarcoma, kaposiform and Dabska-type hemangioendotheliomas, and a subset of angiosarcomas. Mod Pathol 13:180-185, 2000

58. Skobe M, Brown LF, Tognazzi K, et al: Vascular endothelial growth factor-C (VEGF-C) and its receptors KDR and flt-4 are expressed in AIDS-associated Kaposi's sarcoma. J Invest Dermatol 113:1047-1053, 1999

59. Marchiò S, Primo L, Pagano M, et al: Vascular endothelial growth factor-C stimulates the migration and proliferation of Kaposi's sarcoma cells. J Biol Chem 274:27617-27622, 1999

60. Stürzl M, Brandstetter H, Zietz C, et al: Identification of interleukin-1 and platelet-derived growth factor-B as major mitogens for the spindle cells of Kaposi's sarcoma: A combined in vitro and in vivo analysis. Oncogene 10:2007-2016, 1995

61. Ganem D: KSHV-induced oncogenesis, in Arvin A, Campadielli-Fume G, Mocarski E, et al (eds): Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge, MA, Cambridge University Press, 2007